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Accounting for detection unveils the intricacy of wild boar and rabbit co-occurrence patterns in a Mediterranean landscape

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The patterns of species co-occurrence have long served as a primary approach to explore concepts of interspecific interaction. However, the interpretation of such patterns is difficult as they can result from several complex ecological processes, in a scale-dependent manner. Here, we aim to investigate the co-occurrence pattern between European rabbit and wild boar in an estate in Central Portugal, using two-species occupancy modelling. With this framework, we tested species interaction for occupancy and detection, but also the interdependencies between both parameters. According to our results, the wild boar and European rabbit occurred independently in the study area. However, model averaging of the detection parameters revealed a potential positive effect of wild boar's presence on rabbit's detection probability. Upon further analysis of the parameter interdependencies, our results suggested that failing to account for a positive effect on rabbit's detection could lead to potentially biased interpretations of the co-occurrence pattern. Our study, in spite of preliminary, highlights the need to understand these different pathways of species interaction to avoid erroneous inferences.

Understanding the patterns of species co-occurrence is of fundamental interest for many fields of ecological research. Such knowledge underpins much of our understanding of concepts like community assembly¹, competitive exclusion², niche partitioning³ and predator–prey dynamics⁴. In particular, interspecific dependencies in species co-occurrence have served for long as a primary approach to explore concepts related to interspecific interactions^{2,5,6}. The way in which a species occurrence probability is conditioned by the presence or absence of a potential competitor, can serve as a non-mechanistic proxy⁷ for interference competition or facilitation. However, the interpretation of co-occurrence patterns is inherently difficult. Such patterns can result from complex ecological processes acting in tandem, in a scale-dependent manner^{8,9}, or from shared/distinct habitat requirements. For instance, similar habitat preferences could lead to overlapping distributions at a broader scale but, at a patch level, risk/avoidance behaviour of the subordinate species⁹ can be interpreted as spatial displacement. Therefore, at a fine scale, effects of interspecific interaction tend to be more subtle and difficult to demonstrate, as they can manifest in complex ways² (e.g. activity patterns, space use, species abundance). Given the ecological and conservation relevance^{4,6,10} of understanding species co-occurrence patterns, it is important that the complexity underlying these interactions is acknowledged and duly accounted for.

Observational studies to discuss hypotheses on interspecific interactions can be useful when controlled experiments are logistically unfeasible¹¹. Two-species occupancy modelling¹² has emerged as a tool to investigate hierarchical interactions between co-occurring species^{13,14}. Importantly, this method allows to conditionally model the probability of occupancy and detection of a subordinate species upon the presence and/or detection of the dominant species¹². Furthermore, the possibility to include habitat variables¹⁵ allows to interpret such patterns, while accounting for habitat preferences. Past studies provided evidence in support of interspecific dependencies for occupancy and detection probabilities, attributing this to different ecological processes^{4,10,16}. A multi-level approach is important because a fine scale avoidance behaviour by the subordinate species could

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reduce detectability and consequently lead to an overestimation of the spatial exclusion pattern. This is also true if fine scale habitat modifications by the dominant species increase detection of the subordinate species⁸, which would skew inferences of species co-occurrence. However, few studies consider such interdependencies between detection and occupancy parameters¹⁷. Such can be important to unveil new interaction pathways, that would otherwise be masked by unmodelled factors, and/or provide unbiased estimates of the co-occurrence parameter of interest.

European rabbit (*Oryctolagus cuniculus*) and wild boar (*Sus scrofa*) often co-occur with contrasting population densities and a few previous studies^{18–20} investigated a potential interspecific interaction. For the past decades, populations of wild ungulates, such as the wild boar, have been growing all over Europe²¹ and particularly in the Iberian Peninsula²². As a generalist omnivore, the wild boar uses soil rooting to forage. It also wallows in the mud to cool down and remove ectoparasites²³. These behaviours alter soil composition and pH levels, decomposition processes²⁴ and can reduce nearly 80% of the herbaceous cover²⁵, reducing plant diversity and regeneration. These effects on micro-habitat structure may cause the European rabbit to alter the intensity of fine scale space use when wild boar is present, potentially avoiding rooted areas and searching for cover²⁶. Therefore, although co-occurring, signs of presence would be less evident and therefore less detectable. Furthermore, rooting increases soil compaction and nitrogen availability, thus favouring nitrophilous species and reducing leguminous availability²⁷. This affects the rabbit's breeding success since leguminous are essential during this period, and these also constitute an important protein source for wild boar. Moreover, in-depth rooting compromises underground refuge stability (e.g. burrows) for rabbits and there are even some reports of direct predation²⁸. Therefore, direct competition may cause European rabbit to abandon sites less suitable in terms of food and shelter due to wild boar's presence, creating a pattern of spatial displacement.

This potential interspecific competition could further impact already depleted European rabbit populations. These have been declining since the 20th century²⁹, although the species can be locally perceived as a pest within its native range³⁰. Furthermore, it is native to the Iberian Peninsula³¹ and considered a keystone species³². The European rabbit is threatened mainly due to the outbreak of two viral diseases: myxomatosis and viral haemorrhagic disease³³. These exacerbated the on-going decline due to habitat loss and fragmentation^{29,34}, while other relevant threats include predation pressure³⁵, inadequate hunting practices³⁶ and interspecific competition²⁰. More recently, a new variant of the rabbit haemorrhagic disease virus (RHDV2) continues to devastate populations, especially those in low density³⁷. Consequently, the European rabbit is classified as “Nearly Threatened” in Portugal³⁸ and “Vulnerable” in Spain³⁹. However, a recent re-assessment from the IUCN has classified this species as “Endangered”⁴⁰, as the population continues to decline. Therefore, wild boar could hamper the recovery of depleted European rabbit populations. Even more so, given intensive management tends to increase wild boar's numbers as this is a relevant big game species in the Iberian Peninsula⁴¹.

Here we aim to investigate the co-occurrence patterns between the European rabbit and the wild boar in an agroforestry estate in Central Portugal, to elucidate potential interaction pathways. Within a two-species occupancy modelling framework, we specifically evaluated standing expectations that (i) wild boar presence had a negative influence on rabbit's occupancy, (ii) and detection probabilities; and (iii) interdependencies between conditional occupancy and detection probabilities influenced the estimation of co-occurrence patterns. We also included habitat covariates to try to disentangle the effect of wild boar's presence from shared habitat preferences. Testing such hypotheses will further contribute to comprehend the complexity of species co-occurrence patterns and their implications for future research.

Materials and methods

Study area. The study site was the “Charneca”, NE section (100 km²) of Companhia das Lezírias S.A., the largest agroforestry farmstead in Portugal, with nearly 180 km². This area was primarily used for silviculture and pastoral practices. Over the years, different management options shaped a complex landscape, where cork oak Montado was the primary land-cover (~66 km²). The Montado occurred in pure or mixed patches and with variable composition and density of understory, depending on grazing pressures and/or shrub clearance activities. This landscape was interspersed by maritime pine stands, scrublands, olive groves, rice fields and irrigation plots⁴² (Fig. 1).

The study site was also explored as a hunting estate where game species such as the European rabbit and wild boar were targeted. In Companhia das Lezírias' Forest Management reports⁴³, rabbit's local population has been recognised as declining. Since 2008 several conservation measures have been implemented (e.g. scrubland management, food and water supply network, population monitoring) and hunting ceased in 2013. Contrasting, the wild boar has been growing steadily in population numbers and was widespread. As a consequence, the population was controlled through intensive hunting practices⁴³ and during our study, four hunting events occurred (16 December 2015, 20 January 2016, 10 February 2016, 24 February 2016).

Field sampling protocol to assess species presence. We surveyed rabbit and wild boar signs of presence between December 2015 and March 2016. We used a regular sampling strategy by dividing the area into 1 × 1 km plots and prospecting a 25 meters radius buffer around the centre of each plot (i.e. sites, N = 73; Fig. 1). Presence was confirmed by signs such as pellets, latrines and burrows in the case of European rabbit, and droppings, footprints and rooted area in the case of wild boar. Each buffer was prospected by the same two observers, who actively searched for these signs of presence. A species was considered present at a site if at least one of these signs was detected.

We conducted three sampling occasions and during each occasion, all sites (n = 73) were surveyed once, over a period of one to one and a half weeks (\bar{X} = 9 days, SE = 1.528), depending on logistic constraints. Sampling occasions were spaced by a period of two to three weeks. All signs were registered so that in the next sampling occasion we could exclude highly persistent signs such as rabbit burrows, ensuring temporal independence



Figure 1. Location of the study area in Portugal and sampling design in Charneca, Companhia das Lezírias (CL) (map created in QGIS 3.12.0, <http://qgis.osgeo.org>). Depiction of the main land-cover types and the 73 sampling points (sites), 1 km apart, with species- and site-specific detection patterns (open circles for European rabbit, open squares for wild boar, open triangles for both species and closed triangles for neither).

between occasions. Rabbit and wild boar detections were coded as 1 for detected and 0 for non-detected, which allowed us to build site- and species-specific detection histories. For example, the detection history $X_i^A = 001$, means the location i was surveyed on three occasions, with species A being detected only in the last occasion. Overall, we had four sites with missing observations in the last two sampling occasions.

Occupancy modelling. Occupancy models make use of spatial-temporal replicated data to generate a likelihood based estimate using probabilistic arguments that account for false absences^{44,45}. Site- and survey-level covariates can be incorporated when estimating both detection and occupancy parameters via a logit link⁴⁵. Two species occupancy modelling^{12,44} extends this approach allowing the probability of occupancy and detection of a subordinate species to be modelled as a function of the occupancy and detection status of the dominant species. A species interaction factor (SIF) is calculated as a ratio of how likely the two species are to co-occur compared to what would be expected under a hypothesis of independent occupancy¹⁵. A value <1 suggests avoidance, while values >1 indicate co-occurrence. This parameterization assumes: *i*) sites are closed to changes in the occupancy status of the species, *ii*) sites and occasions are, respectively, spatially and temporally independent, and *iii*) there is no un-modelled heterogeneity in both detection and occupancy. Here, we relaxed these assumptions by assuming that, during our sampling period: rabbit populations were stable, as the survey would have ended before the reproductive season⁴⁶, and wild boar's occupancy was interpreted as probability of site use¹², given it is a highly mobile species with large home ranges and likely random movement patterns⁴⁷ within the estate (assumption *i*); furthermore, the time interval between sampling occasions (>2 weeks) mitigated the temporal dependence (assumption *ii*); and the inclusion of detection and occupancy covariates achieved a compromise between model complexity and interpretability (assumption *iii*).

We used a two-stage modelling approach¹⁴: firstly, we used single-species occupancy models to identify relevant covariates for both species and to avoid overparameterization in the next stage¹⁵; secondly, we used two-species models, to assess interspecific interactions. For single-species models, we first tested the effect of the covariates on species detection (p) while keeping occupancy (ψ) constant; then carried the covariate from the best-fitting model and built a second set of candidate models explaining species occupancy probabilities³. For detectability we considered habitat features within the surveyed buffers, but also the rabbit's marking behaviour. Since in our study area European rabbit occurs in low density, large latrines are uncommon⁴⁸ and scattered pellets

were more often used to ascertain species presence. We considered vegetation influenced the detection of such signs and therefore measured the following variables: shrub (S), herbaceous (H), and litter cover (L), shrub (SH) and herbaceous height (HH), and percentage of bare ground (B). For each site ($N = 73$), the same two observers visually estimated an average value of cover percentage and height, during the first sampling occasion. We also used Julian day (JD) as a sampling covariate as the interval between sampling occasions could have influenced species detection.

For occupancy, we selected covariates related to habitat, food availability and disturbance. The wild boar has been described as a generalist species, with preference for forested areas with dense shrub cover and high food availability (e.g. acorns)⁴⁹. Studies on European rabbit established the importance of landscape mosaics of scrublands and pastures⁵⁰. However, the presence of livestock poses a threat to rabbit populations, and ultimately to wild boar, as high grazing pressure leads to shrub clearance and landscape simplification⁵¹. The covariates tested are described in Table 1. These were extracted for a 100 meters radius buffer around each site (according, approximately, to rabbit's largest home-range size in Doñana, Spain⁵²) in software Quantum GIS (QGIS Development Team, 2016), using a GIS database available for the study area³. We mostly tested univariate models, but also a few with covariate combinations representing specific ecological hypothesis (e.g. food and habitat: Mont+Sparse+Cult). We assessed covariate correlation and to avoid multicollinearity excluded the less ecologically meaningful covariate when the coefficient surpassed 0.7⁵³. Herbaceous cover and shrub height covariates were excluded from the analysis (see Supplementary Table S1 online). Also, the dense scrubland covariate led to convergence errors when modelling wild boar's occupancy and was thus removed for this species. Prior to the analysis, all covariates were standardized to z-scores.

In the second stage, we tested for species interaction using the best supported covariates previously identified and following the parameterization of Richmond *et al.* (2010) (see Table 2 for parameter abbreviations). Wild boar ("WB" indicates present; "wb" absent) was considered the dominant species and European rabbit (ER) the subordinate species. To test the hypotheses outlined in the Introduction we built four types of models:

- (i) both occupancy and detection probabilities are unconditional ($\Psi_{WB} \Psi_{ER} p_{WB} p_{ER}$);
- (ii) occupancy is unconditional but detection is conditional ($\Psi_{WB} \Psi_{ER} p_{WB} p_{ER} r_{ER/WB}$);
- (iii) occupancy is conditional but detection is unconditional ($\Psi_{WB} \Psi_{ER/WB} \Psi_{ER/wb} p_{WB} p_{ER}$);
- (iv) both occupancy and detection are conditional ($\Psi_{WB} \Psi_{ER/WB} \Psi_{ER/wb} p_{WB} p_{ER} r_{ER/WB}$).

Model selection. Prior to model ranking we determined the goodness-of-fit of each species global model, using the Pearson chi-square statistic (1000 parametric bootstrap samples). Following the methodology described in MacKenzie and Bailey (2004)⁵⁴, we used the Akaike Information Criterion corrected for small sample size (AICc) for model ranking. This approach assumes that, within the candidate set, models with $\Delta AICc \leq 2$ comparatively to the best model are strongly supported and their covariates are good predictors of the dependent variable. However, the use of an apparently arbitrary cut-off value has been previously criticized, and other values of $\Delta AICc$ of 4, 6 or even 8 may also include relevant models⁵⁵. Thus, for the selection of single-species models we maintained a stricter rule of $\Delta AICc \leq 2$ to avoid overparameterization in the next stage. Then, for the two-species model analysis we resorted to the Akaike's weight (AICw), which indicates the weight of the evidence in favour of a certain model being the most parsimonious in the set. According to Burnham and Anderson (2002)⁵⁶, unless a single model has a $AICw > 0.9$, then other models are likely to be relevant and therefore should also be considered for inference. In the two-species model set, we compared AICw between models with conditional occupancy and/or detection probabilities to compare the evidences supporting our hypothesis.

We used model averaging across the two-species model set to obtain parameter estimates of occupancy and detection probabilities. This also allowed us to compare the different hypothesis, since: if $\Psi_{ER/WB} < \Psi_{ER/wb}$, rabbit's occupancy probability is lower at sites where the wild boar is present, which would indicate spatial displacement ($SIF < 1$). If $p^{ER} > r^{ER}$, rabbit's detection probability is higher when wild boar is absent, and therefore detection is negatively influenced by wild boar's presence. The covariate effect was also ascertained by model averaging. If a covariate was not included in a given model the value was set to zero, and we calculated the weighted average using the respective AICw⁵⁷. For the standard error (SE) we used the formula from Anderson (2008)⁵⁸ and estimated 90% unconditional confidence intervals (CI). We considered a well-supported effect when this interval did not overlap zero. Single-species single-season modelling was done in R statistical software (version 3.4.4) using the "unmarked" package⁵⁹, and two-species single-season modelling was done in software PRESENCE 11.3⁶⁰.

Results

Overall, European rabbit's naïve occupancy (i.e. proportion of sites where species was detected) was 0.36 (26 of the 73 sites), which contrasts with the wild boar with 0.80 naïve occupancy (58 out of 73 sites; Fig. 1). European rabbit and wild boar were detected together in 32% of sites, meaning rabbit was detected alone in only three of the 26 sites it was detected at.

Model averaging of the top-ranking single-species models indicated a well-supported negative effect of the proportion of pine stands on wild boar's occupancy probability and a negative effect of the distance to cultivated areas on European rabbit's occupancy (see Supplementary Tables S2 and S3). Also, litter cover had a negative effect on wild boar's detection probability and shrub cover had a positive effect on rabbit's detection, both well-supported (see Supplementary Tables S3 and S4).

To build the single season two-species candidate model set we combined the four types of models described in the "Occupancy modelling" sub-section, with these four well-supported detection and occupancy covariates. This model set comprised 16 models (see Supplementary Table S5), however, for final inference, we maintained

Covariate	Code	Description	Species
Dense scrubland	Dense	Undisturbed forest and scrubland patches with > 60% understory cover, dominated by <i>Ulex</i> sp., <i>Cistus ladanifer</i> and <i>Cistus monspeliensis</i> .	ER
Sparse scrubland	Sparse	Semi-disturbed forest and scrubland patches, with understory cover between 30–60%, and moderate grazing pressures.	ER/WB
Absent scrubland	Absent	Highly disturbed forest patches with <30% understory cover due to intense grazing pressure and/or shrub clearance activities.	ER/WB
Montado	Mont	Agrosilvopastoral system dominated by <i>Quercus suber</i> , with varied understory densities depending on grazing pressures and shrub clearance activities. Ground mostly covered by natural/permanent pastures.	ER/WB
Pine stands	Pine	Dominated by <i>Pinus pinaster</i> . Stands of varying age, with sparse understory cover.	ER/WB
Landscape heterogeneity	Div	Patch diversity in a 100 meters radius buffer, measured by the Shannon diversity index ($H = -\sum_{i=1}^m P_i \cdot \ln P_i$)	ER/WB
Riparian vegetation	Rip	Dense vegetation adjacent to waterlines, with mixed composition of <i>Salix alba</i> , <i>Fraxinus angustifolia</i> , <i>Alnus glutinosa</i> , <i>Crataegus monogyna</i> and <i>Rubus fruticosus</i> . Measured as the distance, in meters, from the buffer's centroid.	WB
Cultivated areas	Cult	Area dedicated to agricultural practices, composed of olive groves, irrigated areas for forage production and fallow land. Measured as the distance, in meters, from the buffer's centroid.	ER/WB
Artificial feeding	Art	Points of artificial food and water supply for European rabbit. Measured as the distance, in meters, from the buffer's centroid.	ER
Cattle disturbance	Cattle	Grazing pressure index given by $LSU/(ha \times n)$, where LSU is the number of livestock units in a plot of a given area (ha) during a certain number of days (n). Weighted average as a function of the grazing plot area within the 100 meters radius buffer.	ER/WB

Table 1. Covariates selected to model European rabbit (ER) and wild boar (WB) occupancy probability (ψ) in Charneca (CL), according to species' ecological requirements. For each covariate an abbreviation code and brief description are presented.

only the top better fitted 12 models (Table 3). A few of the least supported models had convergence errors and therefore we discarded them, and all inferences were done for this subset that accounts for 90% of the explained variance. From this set, models that support some type of interaction, either for detection, occupancy or both, had a cumulative AICw of 0.569.

Our first hypothesis of a negative effect on European rabbit's occupancy by wild boar's presence was not strongly supported given independence models for occupancy probability ($\psi^{ER/WB} = \psi^{ER/wb}$; AICw = 0.564) out-performed models that indicated spatial displacement ($\psi^{ER/WB} < \psi^{ER/wb}$; AICw = 0.116). Furthermore, the average SIF across the model set supports a scenario of independent occupancy ($SIF = 1.003 \pm 0.114$). Regarding our second hypothesis of wild boar's presence negatively influencing rabbit's detection probability, the evidences supported species interaction ($pER < rER/WB$; AICw = 0.247). For these models, rabbit's detection probability was higher when wild boar was present and averaging across the model set further supported this interaction with estimates of $pER = 0.383 \pm 0.068$ and $rER/WB = 0.489 \pm 0.087$ (Fig. 2).

SIF estimates for models considering rabbit's occupancy conditional upon wild boar's presence exhibited opposite signs depending on the parameterization of rabbit's detection probability. When rabbit's occupancy was conditional upon wild boar's presence, but detection was independent (AICw = 0.322), the models suggested slight co-occurrence ($SIF = 1.067 \pm 0.276$). Models that assumed simultaneously conditional occupancy and detection for rabbit (AICw = 0.116), estimated an avoidance pattern instead ($SIF = 0.845 \pm 0.217$) (Table 3).

In terms of covariate effect, model average of the beta coefficients indicated a positive effect of shrub cover on European rabbit's detection probability ($\beta = 0.158 \pm 0.757$), but the distance to cultivated areas had a negative effect on species occupancy ($\beta = -0.362 \pm 0.484$). For wild boar, detection probability was negatively influenced by litter cover ($\beta = -0.413 \pm 0.43$) and the proportion of pine stands had a negative influence on species occupancy ($\beta = -0.535 \pm 0.639$). However, the large SEs hindered the accurate assessment of the predictors' influence (see Supplementary Table S6), although they had a well-supported effect in the preliminary stage.

Discussion

Our study explored the different pathways of European rabbit and wild boar interaction in an agroforestry farmstead. This fine-scale analysis demonstrated the importance of considering interdependencies between conditional detection and occupancy, an issue often overlooked when unveiling ecological patterns.

The results from occupancy modelling supported our previous knowledge of the study area⁴³: European rabbit had a low occupancy while the wild boar was widespread. Furthermore, the evidences indicated an independent occurrence pattern of European rabbit and wild boar in the study area, which did not support our first hypothesis. Wild boar's wide distribution most likely hindered predictions regarding species co-occurrence, since sites where wild boar was absent were too scarce to draw inferences. Also, the small study area and depleted rabbit population further limited the assessment of species co-occurrence.

Nonetheless, there was some support for interaction in species detection, which indicated that wild boar's presence positively influenced rabbit's detection probability. The model averaged estimates showed that rabbit detection was higher when the wild boar was present, a result that supported our second hypothesis but contrariwise to what was expected. This could have resulted from the combined effect of wild boar's influence on rabbit's behaviour and surveying success. Wild boar's rooting creates open areas which rabbits could have used more intensively for marking⁶¹ and this would have increased detectability. Also, these open areas may increase

Parameter	Definition
ψ_{WB}	Species WB occupancy probability
$\psi_{ER/WB}$	Species ER occupancy probability with species WB present
$\psi_{ER/wb}$	Species ER occupancy probability with species WB absent
p_{WB}	Species WB detection probability with species ER absent
p_{ER}	Species ER detection probability with species WB absent
r_{WB}	Species WB detection probability with species ER present
$r_{ER/WB}$	Species ER detection probability with both species present and species WB detected
$r_{ER/wb}$	Species ER detection probability with both species present and species WB not detected

Table 2. Single-season two-species occupancy model parameters and definition in accordance with the parameterization of Richmond *et al.*, 2010. “WB” stands for wild boar present, “wb” for wild boar absent and “ER” for European rabbit.

Hypothesis	Model	K	AIC	ΔAIC	AICw	SIF
$\Psi(\text{un}), p(\text{un})$	$\psi_{WB}(\text{Pine}) \psi_{ER}(\text{Cult}) p_{WB}(L) p_{ER}(S)$	8	479.82	0	0.183	1
$\Psi(\text{cond}), p(\text{un})$	$\psi_{WB}(\cdot) \psi_{ER/WB}(\text{Cult}) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S)$	8	480.81	0.99	0.112	>1
$\Psi(\text{un}), p(\text{un})$	$\psi_{WB}(\text{Pine}) \psi_{ER}(\cdot) p_{WB}(L) p_{ER}(S)$	7	480.97	1.15	0.103	1
$\Psi(\text{cond}), p(\text{un})$	$\psi_{WB}(\text{Pine}) \psi_{ER/WB}(\text{Cult}) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S)$	9	480.98	1.16	0.103	>1
$\Psi(\text{un}), p(\text{un})$	$\psi_{WB}(\cdot) \psi_{ER}(\text{Cult}) p_{WB}(L) p_{ER}(S)$	7	481.16	1.34	0.094	1
$\Psi(\text{un}), p(\text{cond})$	$\psi_{WB}(\text{Pine}) \psi_{ER}(\text{Cult}) p_{WB}(L) p_{ER}(S) r_{ER/WB}=r_{ER/wb}(\cdot)$	9	481.67	1.85	0.073	1
$\Psi(\text{cond}), p(\text{cond})$	$\psi_{WB}(\text{Pine}) \psi_{ER/WB}(\text{Cult}) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S) r_{ER/WB}=r_{ER/wb}(\cdot)$	10	481.69	1.87	0.072	<1
$\Psi(\text{un}), p(\text{cond})$	$\psi_{WB}(\text{Pine}) \psi_{ER}(\cdot) p_{WB}(L) p_{ER}(S) r_{ER/WB}=r_{ER/wb}(\cdot)$	8	482.12	2.3	0.058	1
$\Psi(\text{cond}), p(\text{un})$	$\psi_{WB}(\text{Pine}) \psi_{ER/WB}(\cdot) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S)$	8	482.16	2.34	0.057	>1
$\Psi(\text{un}), p(\text{un})$	$\psi_{WB}(\cdot) \psi_{ER}(\cdot) p_{WB}(L) p_{ER}(S)$	6	482.31	2.49	0.053	1
$\Psi(\text{cond}), p(\text{un})$	$\psi_{WB}(\cdot) \psi_{ER/WB}(\cdot) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S)$	7	482.42	2.6	0.05	>1
$\Psi(\text{cond}), p(\text{cond})$	$\psi_{WB}(\text{Pine}) \psi_{ER/WB}(\cdot) \psi_{ER/wb}(\cdot) p_{WB}(L) p_{ER}(S) r_{ER/WB}=r_{ER/wb}(\cdot)$	9	482.66	2.84	0.044	<1

Table 3. Model ranking of single season two-species occupancy models testing the different scenarios for species interaction and according to the hypothesis presented. The models with convergence errors were discarded maintaining the sub-set that accounted for a cumulative AICw of 0.9. Model parameters are described in Table 2 and covariate abbreviations in Table 1. “WB” stands for wild boar, “ER” for European rabbit, “un” for unconditional and “cond” for conditional.

observer’s visibility (and thus detection) in areas of dense vegetation. This effect could be greater in low density populations where scattered pellets are more common than big latrines⁴⁸. However, further research into the factors mediating such an interaction, either through behavioural or methodological studies, are necessary.

Assuming this positive effect on rabbit’s detection, we further analysed how this parameter influenced interpretations of species co-occurrence. For models where occupancy was conditioned but detection was not, the pattern was of slight co-occurrence; but for models where both occupancy and detection were conditioned the resulting pattern was of spatial displacement. Although these interaction patterns did not have a strong support, this suggests that failing to account for an interaction in species detection probability could lead to erroneous interpretations of wild boar’s influence on rabbit’s occupancy pattern. Ultimately, if species interaction for occupancy and detection probabilities have opposite signs (positive effect on detection, and negative on occupancy), not accounting for wild boar’s positive effect on detection could lead to interpretations of independent occurrence or even co-occurrence. Furthermore, these interdependencies could contribute to mask similar spatial interactions between species’ pairs⁶², especially for species that affect the habitat’s physical structure^{63,64} and hence potentially influence detectability indirectly. Several authors have demonstrated the importance of considering imperfect detections⁶⁵, but only a few have verified an interaction in detection patterns^{4,17,66}.

When modelling single-species occupancy and detection most of the covariates included in top-ranked models were well-supported. The positive effect of percentage of shrub cover on rabbit’s detection can potentially reflect increased intensity of fine-scale habitat use in more vegetated patches⁵⁰, increasing detectability. Such effect may surpass an anticipated negative influence by reduced visibility of signs of presence. For occupancy, an association to cultivated areas is well documented, since this is an important food resource for rabbit⁶⁷, especially in Mediterranean ecosystems, characterized by strong fluctuations in annual primary production⁶⁸. Wild boar’s detection probability was lower when litter cover was high, probably because during the sampling period, dead leaf cover, especially in Montado, was abundant and could have reduced visibility of signs of presence (i.e. footprints and droppings). A lower occupancy of wild boar in sites with higher proportion of pine stands could be due to the low shrub cover which reduced suitability as a refuge. However, the influence of the landscape variables seems to be unclear, since in the final two-species model framework none of the covariates included had a

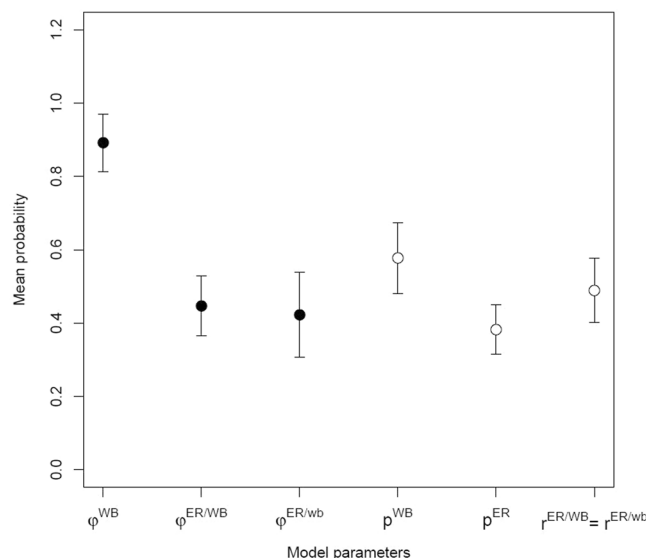


Figure 2. Model average of parameter estimates across the single-season two-species occupancy model set for European rabbit and wild boar, and respective standard error. Occupancy probability estimates (ψ) are represented by closed circles and detection probability estimates by open circles (p). Model parameters are described in Table 2.

well-supported effect. The most likely explanation for this outcome is that the covariates were required because the influence of the other species presence on detection and occupancy was being ignored. Once it was accounted for these covariates' significance was no longer supported.

The small-scale of our study and the current population scenario of European rabbit and wild boar in the area, limited our assessment of a co-occurrence pattern. Therefore, we recommend that field sampling design considers a landscape approach to capture a gradient of wild ungulates' and European rabbits' densities. Also, the use of species abundance instead of binary data could make this pattern more evident at larger scales. Nonetheless, our study was a preliminary approach and resorting to occupancy modelling allowed us to evaluate potential interactions in detection and occupancy, but also the interdependencies between them. The above-mentioned limitations also hindered a definitive assessment of wild boar's effect on rabbit's detection. However, if the suggested positive effect on detection probability is confirmed, then failing to account for it could falsely indicate a co-occurrence pattern. Understanding the reasons behind this interaction requires more in-depth knowledge into European rabbit's fine-scale habitat use. Besides, even if assessing co-occurrence is not of interest *per se*, this means one could overestimate rabbit's presence in areas of wild boar presence.

Therefore, despite the limitations, this complexity of detection and occupancy interdependencies should be acknowledged in future research. Especially, for other wild and domestic ungulates with similar foraging behaviour that creates open areas, potentially influencing rabbit's detection. Furthermore, questions may also arise for any other setting in which the influence of a species promotes the detection of a second one, leading to potential bias if ignored. We reinforce the urgency of research on the potential threat by ungulates and hope that our results and recommendations will better guide future research on this topic. This is particularly relevant since the European rabbit is central to many species conservation plans in Mediterranean ecosystems⁶⁹ and has just recently been reclassified as "Endangered" worldwide by the IUCN⁴⁰.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

- Rich, L. N., Miller, D. A. W., Robinson, H. S., McNutt, J. W. & Kelly, M. J. Using camera trapping and hierarchical occupancy modelling to evaluate the spatial ecology of an African mammal community. *J. Appl. Ecol.* **53**, 1225–1235 (2016).
- Haynes, T. B. *et al.* Occupancy of yellow-billed and Pacific loons: evidence for interspecific competition and habitat mediated co-occurrence. *J. Avian Biol.* **45**, 296–304 (2014).
- Curveira-Santos, G., Marques, T. A., Björklund, M. & Santos-Reis, M. Mediterranean mesocarnivores in spatially structured managed landscapes: community organisation in time and space. *Agric. Ecosyst. Environ.* **237**, 280–289 (2017).
- Murphy, A., Kelly, M. J., Karpanty, S. M., Andrianjakarivelo, V. & Farris, Z. J. Using camera traps to investigate spatial co-occurrence between exotic predators and native prey species: a case study from northeastern Madagascar. *J. Zool.* **307**, 264–273 (2019).
- Farris, Z. J., Kelly, M. J., Karpanty, S. & Ratelolahy, F. Patterns of spatial co-occurrence among native and exotic carnivores in northeastern Madagascar. *Anim. Conserv.* **19**, 189–198 (2016).

6. Santulli, G., Palazón, S., Melero, Y., Gosálbez, J. & Lambin, X. Multi-season occupancy analysis reveals large scale competitive exclusion of the critically endangered European mink by the invasive non-native American mink in Spain. *Biol. Conserv.* **176**, 21–29 (2014).
7. Barner, A. K., Coblenz, K. E., Hacker, S. D. & Menge, B. A. Fundamental contradictions among observational and experimental estimates of non-trophic species interactions. *Ecology* **99**, 557–566 (2018).
8. Sivy, K. J., Pozzanghera, C. B., Grace, J. B. & Prugh, L. R. Fatal attraction? Intraguild facilitation and suppression among predators. *Am. Nat.* **190**, 663–679 (2017).
9. Yackulic, C. B. *et al.* The roles of competition and habitat in the dynamics of populations and species distributions. *Ecology* **95**, 265–279 (2014).
10. Steinmetz, R., Seuaturien, N. & Chutipong, W. Tigers, leopards, and dholes in a half-empty forest: assessing species interactions in a guild of threatened carnivores. *Biol. Conserv.* **163**, 68–78 (2013).
11. Sagarin, R. & Pauchard, A. Observational approaches in ecology open new ground in a changing world. *Front. Ecol. Environ.* **8**, 379–386 (2010).
12. Mackenzie, D. I., Bailey, L. L. & Nichols, J. D. Investigating species co-occurrence patterns when species are detected imperfectly. *J. Anim. Ecol.* **73**, 546–555 (2004).
13. Cruz, J., Sarmiento, P. & White, P. C. L. Influence of exotic forest plantations on occupancy and co-occurrence patterns in a mediterranean carnivore guild. *J. Mammal* **96**, 854–865 (2015).
14. Robinson, Q. H., Bustos, D. & Roemer, G. W. The application of occupancy modeling to evaluate intraguild predation in a model carnivore system. *Ecology* **95**, 3112–3123 (2014).
15. Richmond, O. M. W., Hines, J. E. & Beissinger, S. R. Two-species occupancy models: a new parameterization applied to co-occurrence of secretive rails. *Ecol. Appl.* **20**, 2036–2046 (2010).
16. Wang, Y., Allen, M. L. & Wilmer, C. C. Mesopredator spatial and temporal responses to large predators and human development in the Santa Cruz Mountains of California. *Biol. Conserv.* **190**, 23–33 (2015).
17. Bailey, L. L., Reid, J. A., Forsman, E. D. & Nichols, J. D. Modeling co-occurrence of northern spotted and barred owls: accounting for detection probability differences. *Biol. Conserv.* **142**, 2983–2989 (2009).
18. Cabezas-Díaz, S., Virgós, E., Mangas, J. G. & Lozano, J. The presence of a ‘competitor pit effect’ compromises wild rabbit (*Oryctolagus cuniculus*) conservation. *Anim. Biol.* **61**, 319–334 (2011).
19. Lozano, J., Virgós, E., Cabezas-Díaz, S. & Mangas, J. G. Increase of large game species in Mediterranean areas: is the European wildcat (*Felis silvestris*) facing a new threat? *Biol. Conserv.* **138**, 321–329 (2007).
20. Carpio, A. J., Guerrero-Casado, J., Ruiz-Aizpuru, L., Vicente, J. & Tortosa, F. S. The high abundance of wild ungulates in a Mediterranean region: is this compatible with the European rabbit? *Wildlife Biol.* **20**, 161–166 (2014).
21. Massei, G. *et al.* Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest Manag. Sci.* **71**, 492–500 (2015).
22. Bosch, J. *et al.* Distribution, abundance and density of the wild boar on the Iberian Peninsula, based on the CORINE program and hunting statistics. *Folia Zool.* **61**, 138–151 (2012).
23. Dardaillon, M. Seasonal variations in habitat selection and spatial distribution of wild boar (*Sus scrofa*) in the Camargue, Southern France. *Behav. Processes* **13**, 251–268 (1986).
24. Macci, C. *et al.* Effects of wild boar (*Sus scrofa*) grazing on soil properties in Mediterranean environment. *Catena* **98**, 79–86 (2012).
25. Massei, G. & Genov, P. The environmental impact of wild boar. *Galemys* **16**, 135–145 (2004).
26. Moreno, S., Villafuerte, R. & Delibes, M. Cover is safe during the day but dangerous at night: the use of vegetation by European wild rabbits. *Can. J. Zool.* **74**, 1656–1660 (1996).
27. Carpio, A. J., Oteros, J., Lora, Á. & Tortosa, F. S. Effects of the overabundance of wild ungulates on natural grassland in Southern Spain. *Agrofor. Syst* **89**, 637–644 (2015).
28. Schley, L. & Roper, T. J. Diet of wild boar *Sus scrofa* in Western Europe, with particular reference to consumption of agricultural crops. *Mamm. Rev.* **33**, 43–56 (2003).
29. Delibes-Mateos, M., Ferreras, P. & Villafuerte, R. European rabbit population trends and associated factors: a review of the situation in the Iberian Peninsula. *Mamm. Rev.* **39**, 124–140 (2009).
30. Delibes-Mateos, M. *et al.* A large-scale assessment of European rabbit damage to agriculture in Spain. *Pest Manag. Sci.* **74**, 111–119 (2017).
31. Ferrand, N. & Branco, M. The evolutionary history of the European rabbit (*Oryctolagus cuniculus*): major patterns of population differentiation and geographic expansion inferred from protein polymorphism in *Phylogeography of Southern European Refugia* (eds. Weiss, S. & Ferrand, N.) 207–235 (Springer, 2007).
32. Delibes-Mateos, M., Redpath, S. M., Angulo, E., Ferreras, P. & Villafuerte, R. Rabbits as a keystone species in southern Europe. *Biol. Conserv.* **37**, 149–156 (2007).
33. Calvete, C., Estrada, R., Villafuerte, R., Osácar, J. J. & Lucientes, J. Epidemiology of viral haemorrhagic disease and myxomatosis in a free-living population of wild rabbits. *Vet. Rec.* **150**, 776–782 (2002).
34. Ferreira, C. & Delibes-Mateos, M. Wild rabbit management in the Iberian Peninsula: state of the art and future perspectives for Iberian lynx conservation. *Wildl. Biol. Pract.* **6**, 48–66 (2010).
35. Trout, R. C. & Tittensor, A. M. Can predators regulate wild rabbit *Oryctolagus cuniculus* population density in England and Wales? *Mamm. Rev.* **19**, 153–173 (1989).
36. Angulo, E. & Villafuerte, R. Modelling hunting strategies for the conservation of wild rabbit populations. *Biol. Conserv.* **115**, 291–301 (2004).
37. Guerrero-Casado, J., Carpio, A. J. & Tortosa, F. S. Recent negative trends of wild rabbit populations in southern Spain after the arrival of the new variant of the rabbit hemorrhagic disease virus RHDV2. *Mamm. Biol.* **81**, 361–364 (2016).
38. Queiroz, A. I. *et al.* *Oryctolagus cuniculus* Coelho-bravo in *Livro Vermelho dos Vertebrados de Portugal* (eds. Cabral, M. *et al.*) 279–280 (Instituto da Conservação da Natureza/Assírio & Alvim, 2006).
39. Villafuerte, R. & Delibes-Mateos, M. El conejo in *Atlas y Libro Rojo de los Mamíferos Terrestres de España* 490–491 (2007).
40. Villafuerte, R. & Delibes-Mateos, M. *Oryctolagus cuniculus*. The IUCN Red List of Threatened Species 2019: e.T41291A45189779 (2019). Available at: <https://www.iucnredlist.org/species/41291/45189779>. (Accessed: 11th December 2019).
41. Lopes, F. J. V. & Borges, J. M. F. Wild boar in Portugal. *Galemys* **16**, 243–251 (2004).
42. Gonçalves, P., Alcobia, S., Simões, L. & Santos-Reis, M. Effects of management options on mammal richness in a Mediterranean agro-silvo-pastoral system. *Agrofor. Syst* **85**, 383–395 (2011).
43. Companhia das Lezírias. *Resumo do Plano de Gestão Florestal*. (2016).
44. MacKenzie, D. I. *et al.* *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence* (ed. Maraghioglio, N.) 1–313 (Elsevier, 2006).
45. Mackenzie, D. I. *et al.* Estimating site occupancy rates when detection probabilities are less than one. *Ecology* **83**, 2248–2255 (2002).
46. Beltran, J. Temporal abundance pattern of the wild rabbit in Doñana, SW Spain. *Mammalia* **55**, 591–600 (1991).
47. Massei, G., Genov, P. V., Staines, B. W. & Gorman, M. L. Factors influencing home range and activity of wild boar. *Zool. Soc. London* **242**, 411–423 (1997).
48. Guerrero-Casado, J. *et al.* Assessment of the suitability of latrine counts as an indirect method by which to estimate the abundance of European rabbit populations at high and low abundance. *Eur. J. Wildl. Res.* **66**, 2–5 (2020).

49. Acevedo, P., Escudero, M. A., Muñoz, R. & Gortázar, C. Factors affecting wild boar abundance across an environmental gradient in Spain. *Acta Theriol. (Warsz)* **51**, 327–336 (2006).
50. Lombardi, L., Fernández, N. & Moreno, S. Habitat use and spatial behaviour in the European rabbit in three Mediterranean environments. *Basic Appl. Ecol.* **8**, 453–463 (2007).
51. Moser, B. W. & Witmer, G. W. The effects of elk and cattle foraging on the vegetation, birds, and small mammals of the Bridge Creek Wildlife Area, Oregon. *Int. Biodeterior. Biodegrad.* **45**, 151–157 (2000).
52. Fernández, N. Spatial patterns in European rabbit abundance after a population collapse. *Landsc. Ecol.* **20**, 897–910 (2005).
53. Dormann, C. F. *et al.* Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography (Cop.)*. **36**, 027–046 (2013).
54. Burnham, K. P., Anderson, D. R. & Huyvaert, K. P. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65**, 23–35 (2010).
55. MacKenzie, D. I. & Hines, J. E. Model averaging and individual covariates. *Phidot Forum* (2003). Available at: <http://www.phidot.org/forum/viewtopic.php?f=1&t=996&p=2620&hilit=model+average+betas#p2620>. (Accessed: 18th July 2018).
56. MacKenzie, D. I. & Bailey, L. L. Assessing the fit of site-occupancy models. *J. Agric. Biol. Environ. Stat.* **9**, 300–318 (2004).
57. Burnham, K. P. & Anderson, D. R. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* 1–488 (Springer-Verlag, 2002).
58. Anderson, D. R. Multimodel Inference in *Model Based Inference in the Life Sciences: A Primer on Evidence* 105–124 (Springer Science & Business Media, 2008).
59. Fiske, I. & Chandler, R. Unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. *J. Stat. Softw.* **43**, 1–23 (2011).
60. Hines, J. E. Program PRESENCE 14.3. Software to estimate patch occupancy and related parameters. (2016).
61. Monclús, R. & de Miguel, F. J. Distribución espacial de las letrinas de conejo (*Oryctolagus cuniculus*) en el monte de Valdeletas (Madrid). *Galemys* **15**, 157–165 (2003).
62. Monclús, R., Arroyo, M., Valencia, A. & De Miguel, F. J. Red foxes (*Vulpes vulpes*) use rabbit (*Oryctolagus cuniculus*) scent marks as territorial marking sites. *J. Ethol.* **27**, 153–156 (2009).
63. Cheng, E. & Ritchie, M. E. Impacts of simulated livestock grazing on Utah prairie dogs (*Cynomys parvidens*) in a low productivity ecosystem. *Oecologia* **147**, 546–555 (2005).
64. Thines, N. J. S., Shipley, L. A. & Saylor, R. D. Effects of cattle grazing on ecology and habitat of Columbia Basin pygmy rabbits (*Brachylagus idahoensis*). *Biol. Conserv.* **119**, 525–534 (2004).
65. Kellner, K. F. & Swihart, R. K. Accounting for imperfect detection in ecology: A quantitative review. *PLoS One* **9**, (2014).
66. Olson, C. A., Beard, K. H., Koons, D. N. & Pitt, W. C. Detection probabilities of two introduced frogs in Hawaii: implications for assessing non-native species distributions. *Biol. Invasions* **14**, 889–900 (2012).
67. Barrio, I. C., Bueno, C. G., Villafuerte, R. & Tortosa, F. S. Rabbits, weeds and crops: Can agricultural intensification promote wildlife conflicts in semiarid agro-ecosystems? *J. Arid Environ.* **90**, 1–4 (2013).
68. Calvete, C., Estrada, R., Angulo, E. & Cabezas-Ruiz, S. Habitat factors related to wild rabbit conservation in an agricultural landscape. *Landsc. Ecol.* **19**, 531–542 (2004).
69. Palomares, F., Rodríguez, A., Revilla, E., Vicente López-Bao, J. & Calzada, J. Assessment of the conservation efforts to prevent extinction of the Iberian lynx. *Conserv. Biol.* **25**, 4–8 (2010).

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Author contributions

A.L.B. conceived the study with contributions from T.A.M., G.C.S. and M.S.R. A.L.B. collected the data. A.L.B., T.A.M. and G.C.S. analysed the data. A.L.B. led the writing of the manuscript and prepared the figures and tables. All authors contributed to the drafts and gave final approval for publication.

Competing interests

The authors declare no competing interests.

Additional information

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